Sobia Kauser[1], Gillian E. Westgate[1,2], Martin R. Green[3] and Desmond J. Tobin[1]

[1]Centre for Skin Sciences, School of Life Sciences, University of Bradford, Bradford, West Yorkshire, UK; [2]Westgate Consultancy Ltd, Stevington, Bedford, UK and [3]Unilever R&D Colworth Laboratory, Sharnbrook, Bedford, UK
E-mail: d.tobin@bradford.ac.uk

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Full Sequencing of the *FLG* Gene in Italian Patients with Atopic Eczema: Evidence of New Mutations, but Lack of an Association

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TO THE EDITOR

Atopic eczema (AE) (OMIM %603165) is the most common chronic inflammatory skin disease, characterized by xerosis, pruritus, and erythematous lesions with increased transepidermal water loss. In recent years, it has been suggested that the epidermal skin barrier has a significant role in AE disease susceptibility and severity (Smith *et al.*, 2006; Cork *et al.*, 2009). Sandilands *et al.* (2006) demonstrated that null mutations within the filaggrin gene (*FLG*) strongly predispose individuals to AE. Two *FLG* null alleles (R501X and 2282del4) have been shown to be significantly associated with AE in several European populations (Palmer *et al.*, 2006; Weidinger *et al.*, 2007). Recently, a meta-analysis of the most common *FLG* variants in European populations, involving 5,791 eczema cases and 26,454 controls (Rodriguez *et al.*, 2009), revealed that there is a high risk conferred by R501X and 2282del4 across the studies, with an overall odds ratio of 3.14 and 2.78, respectively. Indeed, large differences in carrier frequencies exist across Europe, ranging from 1.4% in an Italian population (Giardina *et al.*, 2008) to 63% in an Irish population (Palmer *et al.*, 2006). Recently, we observed that in Italian patients the frequencies of R501X and 2282del4 are strongly reduced with respect to those described in other patients of European origin, and the frequencies are similar between cases and controls (0.6 vs. 0.0% and 0.9 vs. 0.5%, respectively). In order to determine whether other mutations located elsewhere in *FLG* confer risk to AE, we performed a full sequencing of *FLG* in Italian patients.

We performed a sequencing of the full *FLG* gene in a cohort of 220 Italian AE patients (recruited by IDI-Istituto Dermopatico dell'Immacolata and Fatebenefratelli Hospital). We then determined the frequency of variations and mutations in a cohort of 201 healthy subjects. The diagnosis of AE in our case cohort was made by experienced dermatologists or by a pediatric allergologist. The cohort consisted of 85% of cases with the intrinsic subtype and 15% with the extrinsic form of AE. These subtypes and severity of AE have been established based on the total IgE level (extrinsic subtype $>500\,\mathrm{ng\,l^{-1}}$) and using the scoring atopic dermatitis. Further clinical details of Italian patients are available in

Abbreviations: AE, atopic eczema; FLG, *filaggrin gene; LD, linkage disequilibrium*

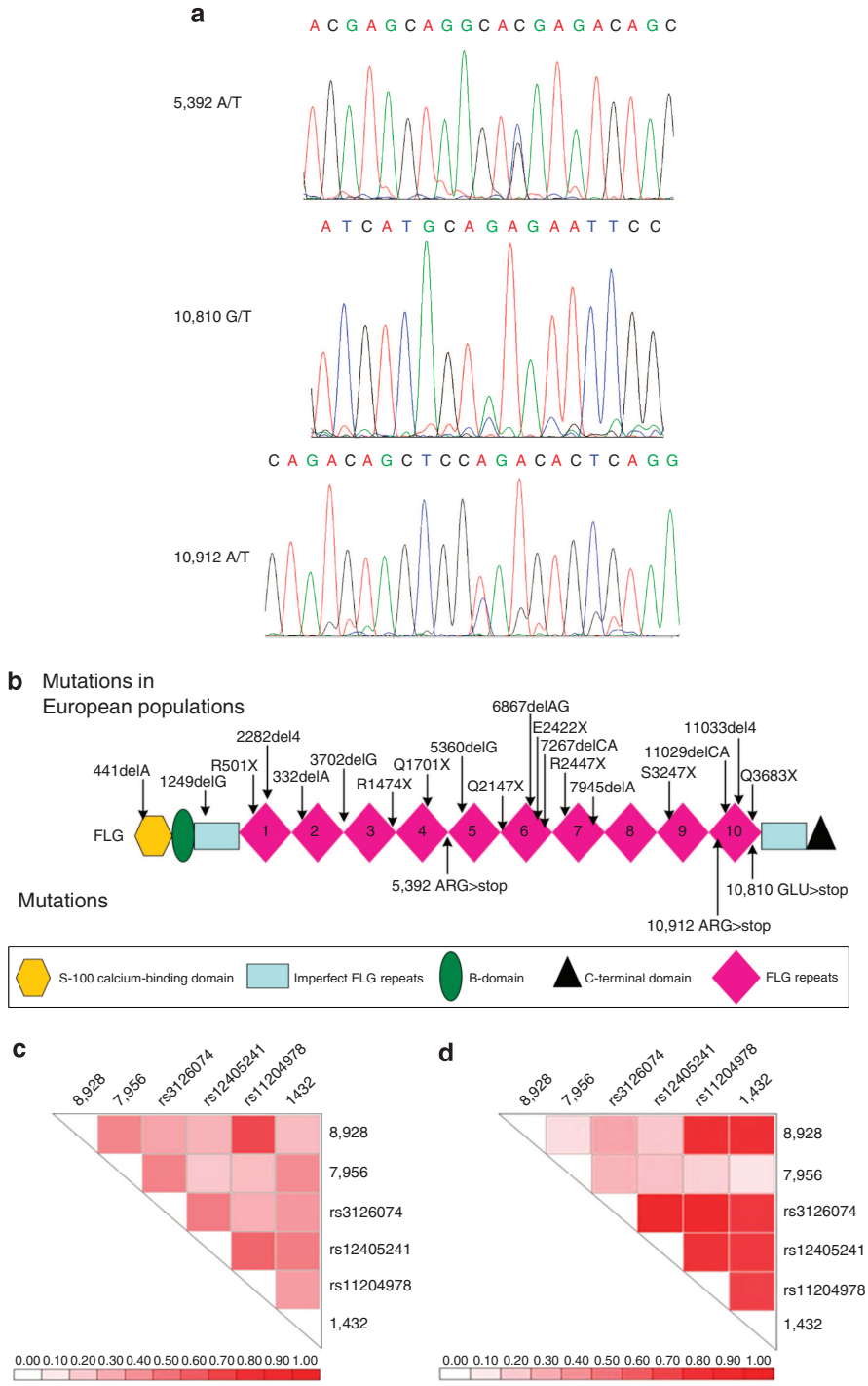

Figure 1. *FLG* null mutations and linkage disequilibrium (LD) plot. (**a**) Electropherograms of the null mutations. (**b**) Gene location of filaggrin (*FLG*) null mutations. (**c**) LD pattern in atopic eczema cases. (**d**) LD pattern in healthy controls.

Bergboer *et al*. (2010). Written consent was obtained from all of the patients or their parents. The study was approved by the ethics committee of each participating institute and complied with the guidelines of the Declaration of Helsinki.

Genomic DNA was extracted from peripheral blood using standard procedures. Sequence analysis of the third exon of *FLG* using 11 overlapping fragments amplified by 11 sequence-specific primer pairs was performed (see Supplementary Table S1 online). Furthermore, the promoter region and exon 2 were also analyzed. PCR fragments were run on an ABI 3130xl automated sequencer (Applied Biosystems, Foster City, CA). We identified

Table 1. Allele frequencies of three unreported mutations

	R1798X_C>T		E3603X_G>T		R3638X_A>T	
Allele	**C**	**T**	**G**	**T**	**A**	**T**
Cases	0.998	0.002	0.995	0.005	0.97	0.03
Controls	0.997	0.003	1	0	0.96	0.04

three null mutations that, to our knowledge, are previously unreported (R1798X (5392 C>T), E3603X (10810 G>T), and R3638X (10912 A>T)) (Figure 1a and b) and are located in the repeats 5 (R1798X) and 10 (E3603X and R3638X), adjacent to the other null mutations identified thus far. Subsequently, we assessed the frequency of the mutations in 201 healthy controls. We found that the frequency of the mutations was similar in the cases and controls (Table 1). The mutations R1798X and E3603X are rare in an Italian population, and, although not statistically significant, further studies in Italians, as well as other populations, will be needed to confirm their frequency and/or rule out a functional role. The null mutation, R3638X, has a higher frequency in healthy controls (4%) and might be considered a polymorphic variant. Moreover, we identified a number of variations, but failed to observe evidence of an association in our patients. Therefore, it is likely that in Italian cases there are other genes associated with the 1q21 region (ATOD2). In this regard, we believe that a study involving Italians, as well as other 1q21-linked samples, can be particularly useful to detect population-specific associated alleles. As in a critical region that harbors the susceptibility variant the extent of linkage disequilibrium (LD) may be different between cases and controls, we decided to contrast LD patterns between AE cases and controls (Figure 1c and d). We considered all the variations with a frequency >0.1 at the *FLG* locus and found a different pattern of LD. In particular, we observed a more conserved block between markers rs12405241 and 1432 in controls with respect to the cases. Although these data need to be replicated in other populations and using a higher number of markers, it is suggested that disease-bearing chromosomes and control chromosomes arise from different pools, with disease-bearing chromosomes showing greater heterogeneity. Further studies will be needed to verify the existence of multiple susceptibility alleles spanning the ATOD2 locus.

Taken together, these results demonstrate that only a small percentage of Italian AE patients carry mutations in the *FLG* gene, which therefore should not be considered as a major risk factor for AE in the Italian population. The low frequency of *FLG* null alleles and the absence of association in the AE cohort may reflect the strong purifying selection of *FLG* mutations in chromosome 1 of Italian patients. In this regard, it is notable that the frequency of mutations is strongly reduced in healthy controls with respect to that observed in different European populations, further supporting the existence of a different ethnic origin of Italian population and ruling out a possible confounding effect of clinical and genetic heterogeneity characterization of samples. An alternative explanation would be that the mutations are strongly advantageous in northern Europe. As a result, the geographic distribution of the frequencies of *FLG* mutations is heterogeneous among European populations. Currently there is no evidence of *FLG* mutations in Mediterranean populations, making possible the existence of a population-specific gradient across Europe. With this perspective in mind, a pan-European study of *FLG* mutations should be conducted to further address this hypothesis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

Raffaella Cascella[1], Valeria Foti Cuzzola[2], Tiziana Lepre[1], Elena Galli[3], Viviana Moschese[4], Loredana Chini[4], Cinzia Mazzanti[5], Paola Fortugno[6], Giuseppe Novelli[1,7,8,9] and Emiliano Giardina[1]

[1]Centre of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases, School of Medicine, Tor Vergata University of Rome, Rome, Italy; [2]IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy; [3]San Pietro Hospital, Fatebenefratelli, Rome, Italy; [4]Department of Pediatrics, Tor Vergata University, Rome, Italy; [5]Day Hospital Service, II Dermatology Division, Istituto Dermopatico dell'Immacolata, IDI-IRCCS, Rome, Italy; [6]Laboratory of Molecular and Cell Biology, Istituto Dermopatico dell'Immacolata, IDI-IRCCS; [7]Department of Cardiovascular Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; [8]Department of Dermatology, Tor Vergata Universtiy of Rome, Rome, Italy and [9]Department of Applied Clinical and Medical Therapy, Rheumatological Unit, "La Sapienza" University of Rome, Rome, Italy
E-mail: emiliano.giardina@uniroma2.it

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